THE EFFECT OF PNEUMATIC TOURNIQUETS ON THE ULTRASTRUCTURE OF SKELETAL MUSCLE

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Experiments have been carried out on rhesus monkeys to determine the effect of the application of a pneumatic tourniquet on the ultrastructure of the muscles of the lower limb. Tourniquets were applied for periods lasting between one and five hours. The changes in the muscle lying immediately under the cuff of the tourniquet were more marked than those observed in muscle distal to the cuff. Three hours appears to be close to the limit of the time that a muscle can resist the sustained compression of a tourniquet.

The length of time that it is safe to leave a tourniquet in place on a healthy limb without causing irreversible damage to the skeletal muscle is of importance in orthopaedic practice. Present-day recommendations, based mainly on personal experience, vary from one hour (Rank, Wakefield and Hueston 1968) to two hours (Boyes 1964) with an upper limit at three hours (Parkes 1973). Compression and ischaemia are the factors most likely to contribute to the muscular damage. Changes in the muscle resulting from tourniquet-induced ischaemia have been studied from many aspects: histological (Harman and Gwinn 1949; Dahlbäck 1970), histochemical (Moore, Ruska and Copenhaver 1956; Dahlbäck 1970; Tountas and Bergman 1977), biochemical (Harman and Gwinn 1949) and ultrastructural (Moore et al. 1956; Strock and Majno 1969; Tountas and Bergman 1977). However, the effect of compression on the muscle lying immediately under the tourniquet has received little attention.

Mammalian muscle is composed of three main types of fibre, termed fast-twitch white, fast-twitch red and slow-twitch intermediate (Barnard et al. 1971). The fast-twitch red and slow-twitch intermediate fibres rely primarily on oxidative metabolism and are more resistant to fatigue than the mainly glycolytic fast-twitch white fibres. Using two muscles, the soleus and the extensor digitorum longus, which between them contain representatives of all three types of fibre, the possibility of a differential response to ischaemia has been investigated.

MATERIALS AND METHODS

Adult rhesus monkeys weighing from 3.5 to 5 kilograms were used. After a preliminary injection of ketamine hydrochloride (10 milligrams per kilogram) anaesthesia was induced with nitrous oxide, halothane and oxygen. The animal was intubated and an intravenous drip (0.18 per cent saline, 4 per cent dextrose) was set up for the duration of anaesthesia. A Kidde tourniquet cuff of infant size was applied to the upper thigh of the right lower limb for periods lasting from one to five hours at a pressure of 300 millimetres of mercury. Immediately before the release of the tourniquet, samples from the soleus and the extensor digitorum longus were removed for biopsy and processed for electron microscopy. Samples from the muscle lying under the tourniquet, the quadriceps, were taken after removal of the tourniquet.

Recovery from three-hour and five-hour tourniquets was investigated. Samples from the quadriceps, extensor digitorum longus and soleus were taken one day, two or three days and seven days after release of the tourniquet. Only one sample was removed from any one particular muscle since in trial experiments repeated sampling in itself was found to cause marked damage to the fibres. Samples from the opposite limb were used as controls.

Electron microscopy. Immediately after biopsy, bundles of muscle fibres were pinned to a cork in a slightly stretched condition. They were then immersed in 3 per cent glutaraldehyde buffered with 0.1M cacodylate buffer, pH 7.3, and fixed for one hour at room temperature and for a further hour after the fibres had been separated into smaller bundles. Next, the fibres were given several washes in cacodylate buffer containing 5 per cent sucrose and then post-fixed for one hour on ice in 1 per cent osmium tetroxide buffered with 0.1M cacodylate buffer, pH 7.3. After being washed in distilled water, the fibres were dehydrated in acetone and then in propylene oxide and subsequently embedded in Spurr's epoxy resin. Transverse and longitudinal sections were cut on a Reichert ultramicrotome and collected on copper grids. Sections were stained first with uranyl acetate dissolved to saturation point in ethanol and then in Reynolds' lead citrate solution. They were coated with carbon and examined in a Philips EM 300 electron microscope. A minimum of fifteen fibres from each sample of muscle were examined and photographed.

RESULTS

Effect of ischaemia on the extensor digitorum longus and soleus muscles

The extensor digitorum longus is composed of fast-twitch white and fast-twitch red fibres. These were differentiated by the thicker Z lines and more abundant mitochondria of the fast-twitch red fibres (Fig. 1). Fast-twitch red and slow-twitch intermediate fibres
make up the soleus muscle. Again, these two fibres were differentiated by the thicker Z lines of the fast-twitch red fibres. Differences in mitochondrial content are not great enough to allow these two types of fibre to be distinguished in transverse sections.

After one hour of ischaemia marked changes in mitochondrial morphology were observed in the fibres of both the extensor digitorum longus and the soleus: they had become swollen, less electron-dense and had lost their organised network of cristae but many fibres displaying normal mitochondrial morphology were still present. As the period of ischaemia was progressively increased up to five hours, a greater percentage of the fibres showed mitochondrial damage, which was similar in all three types of fibre (Figs. 2 and 3). Except for a reduction in the number of granules of glycogen in the fast-twitch red and fast-twitch white fibres, ischaemia had no immediate effect on any other component of the fibres.

Recovery of the extensor digitorum longus and soleus from a three-hour tourniquet was rapid. One day after release of the tourniquet the majority of fibres in both muscles appeared normal. Fibres with spaces, usually at the level of the 1 band, were occasionally encountered. Frequently these spaces contained membranous material which probably represented the remains of degenerating mitochondria. The levels of glycogen had returned to those found in control fibres.

**Fig. 1**
Longitudinal sections through the normal extensor digitorum longus. Note the difference in thickness between the Z discs of the fast-twitch white fibre (top arrow) and the fast-twitch red fibre (bottom arrow). (x 30 800.)

**Fig. 2**
Muscle sampled immediately after an ischaemic period of three hours. Figure 2—Longitudinal sections through the soleus. Swollen mitochondria which have lost their organised array of cristae are present in both the slow-twitch intermediate fibre (top arrow) and the fast-twitch red fibre (bottom arrow). (x 34 600.) Figure 3—Transverse section through the extensor digitorum longus. Swollen mitochondria which have lost their organised array of cristae (arrows) are observable in both fast-twitch red fibres (R) and fast-twitch white fibres (W). (x 7800.)
A similar result was obtained in the soleus one day after a five-hour tourniquet, but in the extensor digitorum longus, infiltrating polymorphs and damaged and normal fibres were found. The damaged fibres had enlarged mitochondria, the Z discs were eroded away and electron-dense deposits were found interspersed between the myofibrils (Fig. 4). Except for a few fibres containing the remnants of degenerating mitochondria, the majority of fibres from both muscles had totally recovered three days after a five-hour tourniquet. The extensive damage found in the extensor digitorum longus one day after a five-hour tourniquet was not observed in any of the fibres examined. After seven days all the fibres that had been subjected to a five-hour period of ischaemia were indistinguishable from those of the control muscles.

**Effect of tourniquet pressure on the quadriceps muscle**

**Three-hour tourniquet.** All muscles both immediately and twenty-four hours after removal of the tourniquet were normal except for a slight swelling of the mitochondria. In one experiment in which the muscle was examined two days after the release of the tourniquet, approximately 50 per cent of the fibres were swollen and the cristae disorganised.

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**Fig. 4**
Longitudinal section through the extensor digitorum longus one day after release from a five-hour tourniquet. The Z discs are eroded away (arrows) and a polymorph is present in the inter-fibre space. (× 8650.)

**Fig. 5**
Transverse section through the quadriceps two days after release from a three-hour tourniquet. Figure 5—The myofibrils are disoriented and electron-dense products are present in the mitochondria (arrow). (× 30,800.) Figure 6—A polymorph can be observed penetrating between the myofibrils. (× 12,800.)

**Fig. 6**

**Fig. 7**
Transverse section through the quadriceps on release of a five-hour tourniquet. The mitochondria are swollen and their cristae disorganised. (× 12,800.)
showed significant changes. The I and Z bands were lost and the remaining A bands were frequently disoriented, so that filaments sectioned in a longitudinal plane lay adjacent to others cut transversely. The mitochondria of these fibres often contained electron-dense products (Figs. 5 and 6). Polymorphs were found in the inter-fibre spaces and sometimes penetrating between the myofibrils (Fig. 6). All muscle fibres examined seven days after the release of a three-hour tourniquet were morphologically identical to those of control fibres.

**Five-hour tourniquet.** Muscle samples from the quadriceps taken immediately after the release of a five-hour tourniquet showed extensive mitochondrial damage similar to that observed in the ischaemic extensor digitorum longus and soleus. In addition there was slight filament erosion at the Z and I band levels and the sarcolemma was broken and fragmented (Fig. 7). One day after the release the Z disc was totally eroded from all the fibres examined (Figs. 8 and 9). The thin actin filaments no longer appeared rigid and straight but ran a spidery path into the A band. The mitochondria still remained swollen and pale and the sarcolemma was fragmented (Fig. 9). Polymorphs (Fig. 9) and red cells were frequently found in the inter-fibre spaces.
On the third day after the release of the tourniquet many totally necrotic fibres were found. These fibres were filled with amorphous material and did not show the characteristic areas of A, I and Z bands (Fig. 10). Fibres with intact filament and triad systems were also common at this stage of recovery. However, such fibres often contained large myelin figures (Fig. 11). Fibroblast cells lying between the fibres were occasionally encountered.

Approximately two-thirds of the fibres had intact contractile filament and triad systems seven days after the release of the tourniquet. Dense amorphous material was found lying between the myofibrils in a small number of these fibres (Fig. 12). The remaining fibres appeared to be engaged in resynthesising contractile material (Fig. 13). The nuclei of these fibres often occupied a more central position and stretches of endoplasmic reticulum were dispersed throughout their cytoplasm.

**DISCUSSION**

Previous studies have been primarily concerned with the effects of ischaemia on those muscles distal to the tourniquet (Harman and Gwinn 1949; Moore et al. 1956; Dahlbäck 1970; Tountas and Bergman 1977). The results presented here suggest that, in addition to ischaemia, the effects of compression require consideration. Tourniquets applied for long periods caused more severe and lasting damage to the muscle lying beneath the tourniquet than to muscles lying distal to it. The sarcolemmal damage observed in fibres of the quadriceps immediately after removal of a five-hour tourniquet would have detrimental effects on their excitation-contraction coupling system. The total erosion of the Z discs found twenty-four hours after removal of a five-hour tourniquet would render the development of tension impossible in these fibres. Although fibres with a reasonably normal structure were found on the third and seventh days after the release of a five-hour tourniquet, the general ultrastructural picture was still aberrant. Even fibres with intact contractile systems were occasionally found to have deposits of amorphous material lying between the myofibrils. The nature of this amorphous material is not known.

The mechanism of recovery from pressure was not identified in the present study. Myotubes, with their characteristic lines of centrally positioned nuclei which have been described in other studies on regeneration of muscle (Allbrook 1962), were not encountered. However, fibres which appeared to be in the process of resynthesising new myofibrillar material were found. The nuclei of such fibres often occupied a central position in the fibre. Regeneration of muscle may involve destruction of damaged fibres and the formation of new ones. Alternatively, damaged fibres may be repaired directly. The penetration of damaged fibres by polymorphs would suggest the first of these mechanisms. However, normal fibres were found three days after a five-hour tourniquet; this time is insufficient to allow for the destruction of fibres and formation of new ones. Both mechanisms may therefore be operative, the regenerative route perhaps depending on the extent of damage. Further studies are being carried out to elucidate the finer details of the regenerative process.

The time a tourniquet is left in place appears to be a critical factor in determining whether or not severe damage occurs to the underlying muscle. After five hours, there was evidence of severe damage in all the muscle samples subsequently examined. On the other hand, only one of four monkeys showed any sign of severe damage after a three-hour tourniquet. It may be that three hours is close to the limit of the time that a muscle can resist sustained compression and that the muscles of more susceptible individuals succumb after this period. Although a number of investigators have
reported the effect of ischaemia on muscles distal to the tourniquet, their findings have not always been uniform. There is general agreement that the mitochondria swell and the cristae become disorganised. Tountas and Bergman (1977), working with cynomolgus monkeys, found that the mitochondria were the only component of the muscle fibre to undergo change, and seven days after the release of the tourniquet the muscle was normal. Dissolution of Z discs was observed sixteen hours after two-hour tourniquets were released from the limbs of mice (Moore et al. 1956). In studies in the rabbit, tourniquets applied for times as short as thirty minutes resulted in degenerating fibres and infiltrating phagocytic cells, visible with the light microscope, being found one day later (Dahlbäck 1970).

In our experiments with rhesus monkeys, the mitochondria were the only components of the extensor digitorum longus and soleus to show any significant degree of damage after a three-hour tourniquet. These organelles seem to be very sensitive to ischaemia since changes were observed after only one hour. In studies on ischaemic heart muscle, mitochondrial damage was observed after as little as twelve minutes (McAllister, Munger and Neely 1977). The soleus and extensor digitorum longus of the rhesus monkeys showed a remarkable ability to regenerate their mitochondria. Mitochondrial structure was normal three days after a three-hour period of ischaemia and seven days after a five-hour ischaemia. It was interesting to find normal fibres interspersed with others showing mitochondrial damage. Moore et al. (1956) have reported similar observations in ischaemic muscle in mice. Resistance or susceptibility of the mitochondria of muscle fibre to ischaemia does not appear to be related to the type of fibre since similar changes were observed in all three types. The metabolism of fast-twitch red and slow-twitch intermediate fibres is primarily oxidative while the fast-twitch white fibres are mainly glycolytic. Physiologically this may be manifested in the former two types giving earlier fatigue times.

The more severe effects of ischaemia observed by some workers may be related to the species of animal studied. In the rhesus monkey a three-hour tourniquet did not result in damage to the contractile filaments. However, in one monkey subjected to a five-hour tourniquet, infiltrating cells and eroded Z discs were found in the extensor digitorum longus one day after tourniquet release. This may indicate that five hours of ischaemia is close to the limit of time that the muscle can endure without becoming severely damaged.

The contractile machinery of the muscle fibres of the rhesus monkey appeared to be wholly unaffected by periods of ischaemia lasting up to three hours. However, it is important to correlate these morphological results with the appropriate physiological experiments. Such experiments are planned. From the viewpoint of the practising surgeon it seems clear that in considering safety factors when a tourniquet is applied, the effect on the muscle beneath the tourniquet cuff is more significant than that on the muscle distal to the cuff. With unduly high pressures this effect may be produced more rapidly and hence the lowest effective pressure that produces a satisfactory bloodless field should be used (Klenerman and Hulands 1979). More detailed studies are necessary to assess the effect of variation of pressure in the production of local damage.

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REFERENCES


